

WHAT IS CLAIMED:

1. A method of screening for compounds that modulate sweet taste signaling comprising:
 - (i) contacting a cell co-expressing at least two T1R receptors or polypeptides on its surface with a putative taste modulating compound; and
 - (ii) measuring activity from the T1R receptors or polypeptides expressed on the cell surface wherein the expressed T1R receptors or polypeptides act a hetero-oligomeric complex.
- 10 2. The method of claim 1, wherein the T1R receptor or polypeptide activity is measured by assayed by measuring changes in intracellular Ca^{2+} levels, cAMP, cGMP and IP3, or G protein binding of GTP γ S.
- 15 3. The method of claim 1, wherein the cell is transfected with at least one additional nucleic acid construct encoding a gene involved in taste signaling.
- 20 4. The method of claim 3, wherein said at least one additional gene encodes a G protein involved in taste signal transduction.
5. The method of claim 4, wherein said G protein is a promiscuous G protein.
- 25 6. A method of screening for compounds that modulate taste signaling transduction comprising:
 - (i) contacting a cell co-expressing at least two T1R receptors or polypeptides with a known taste activating compound and a compound putatively involved in taste transduction modulation, wherein the expressed T1R receptors or polypeptides act as a hetero-oligomeric complex;
 - (ii) contacting a second cell co-expressing at least two T1R receptors or polypeptides with a known taste activating compound alone, wherein the expressed T1R receptors or polypeptides act as a hetero-oligomeric complex; and

(iii) comparing the activity from the T1R receptors or polypeptides expressed on the cell surface of the cell of step (i) with the activity from the T1R receptors or polypeptides expressed on the cell surface of the cell of step (ii) to identify modulators of taste transduction.

5

7. The method of claim 6, wherein said modulatory compounds are selected from the group consisting of activators, inhibitors, stimulators, enhancers, agonists and antagonists.

10

8. The method of claim 6, wherein T1R receptor or polypeptide activity is measured by assayed by measuring changes in intracellular Ca^{2+} levels, cAMP, cGMP and IP3, or G protein binding of GTP γ S.

15

9. The method of claim 6, wherein said cell is transfected with at least one additional nucleic acid construct encoding a gene involved in taste signaling.

10. The method of claim 9, wherein said at least one additional gene encodes a G protein involved in taste signal transduction.

20

11. The method of claim 10, wherein said G protein is a promiscuous G protein.

25

12. An isolated nucleic acid molecule encoding a G protein-coupled receptor polypeptide active in taste signaling comprising the nucleotide sequence of SEQ ID NO: 1.

13. An isolated nucleic acid molecule encoding a G protein-coupled receptor polypeptide active in taste signaling comprising the nucleotide sequence of SEQ ID NO: 3.

30

14. An isolated nucleic acid molecule encoding a G protein-coupled receptor polypeptide active in taste signaling comprising the nucleotide sequence of SEQ ID NO: 5.

5 15. An isolated nucleic acid molecule encoding a G protein-coupled receptor polypeptide active in taste signaling comprising the nucleotide sequence of SEQ ID NO: 7.

10 16. An isolated nucleic acid molecule encoding the G protein-coupled receptor polypeptide active in taste signaling comprising the amino acid sequence of SEQ ID NO: 2.

15 17. An isolated nucleic acid molecule encoding the G protein-coupled receptor polypeptide active in taste signaling comprising the amino acid sequence of SEQ ID NO: 4.

20 18. An isolated nucleic acid molecule encoding the G protein-coupled receptor polypeptide active in taste signaling comprising the amino acid sequence of SEQ ID NO: 6.

25 19. An isolated polypeptide selected from the group consisting of:
(i) a G protein-coupled receptor polypeptide active in taste signaling encoded by a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS 1, 3, 5, and 7;
(ii) a G protein-coupled receptor polypeptide active in taste signaling comprising an amino acid sequence selected from the group consisting of SEQ ID NOS 2, 4, and 6;
(iii) a G protein-coupled receptor polypeptide active in taste signaling encoded by a nucleic acid molecule comprising a nucleic acid sequence having at least 30 about 50% identify to a nucleic acid sequence selected from the group consisting of SEQ ID NOS 1, 3, 5, and 7;

(iv) a G protein-coupled receptor polypeptide active in taste signaling comprising an amino acid sequence that is at least about 40% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs 2, 4, and 6;

(v) a variant of a G protein-coupled receptor polypeptide active in taste signaling encoded by a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs 1, 3, 5, and 7, wherein said variant protein contains at least one conservative substitution relative to the G protein-coupled receptor encoded by said nucleotide sequence; and

(vi) a variant of a G protein-coupled receptor polypeptide active in taste signaling comprising an amino acid sequence selected from the group consisting of SEQ ID NOS 2, 4, and 6, containing at least one conservative substitution.

20. A fragment of the polypeptide of claim 19, wherein said fragment comprises at least about 5 to 7 amino acids.

15

21. The fragment of claim 20, wherein said fragment contains an extracellular domain of a T1R mammalian G protein-coupled receptor polypeptide.

20

22. The fragment of claim 21, wherein said extracellular domain interacts with a compound involved in taste activation or modulation.

23. The fragment of claim 21, wherein said extracellular domain interacts with a protein involved in taste signal transduction.

25

24. The fragment of claim 23, wherein said protein involved in taste signal transduction is a G protein subunit.

25. The fragment of claim 23, wherein said G protein subunit is a promiscuous G protein.

30

26. The fragment of claim 23, wherein said protein involved in taste signal transduction is another T1R polypeptide.

27. The fragment of claim 20, wherein said fragment includes at least an N-terminal fragment of T1R receptor.

5 28. The fragment of claim 27, wherein said N-terminal fragment is involved in ligand binding.

10 29. The polypeptide fragment of claim 20, wherein said fragment is at least about 100 amino acids in length.

15 30. The polypeptide fragment of claim 20, wherein said fragment is at least about 600 amino acids in length.

20 31. A chimeric or fusion polypeptide comprising at least part of the amino acid sequence of a polypeptide of claim 19, and at least part of a heterologous amino acid sequence.

25 32. The chimeric or fusion polypeptide of claim 31, wherein said heterologous sequence is a sequence from a different G protein-coupled receptor.

30 33. The chimeric or fusion polypeptide of claim 32; wherein said heterologous sequence is a sequence from green fluorescent protein.

35. A polypeptide array comprising at least about a 5 to 7 amino acid segment of at least two polypeptides according to claim 19, wherein said at least two polypeptide segments are linked covalently or noncovalently to a solid phase support and the polypeptide segments act as a hetero-oligomeric complex..

30 35. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

100-200-300-400-500-600-700-800-900-1000

36. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 4.

37. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 6.

38. A method of screening one or more compounds for the presence of a compound that activates or modulates sweet taste signaling, comprising contacting said one or more compounds with one or more fragments of at least two polypeptides according to claim 19, wherein the one or more fragments are at least about a 5 to 7 amino acids in length and the at least two polypeptides act as a hetero-oligomeric complex.

39. A biochemical assay for identifying tastant ligands having binding specificity for G protein-coupled receptors active in taste signaling, comprising:

(i) contacting at least two fragments according to claim 20 with one or more putative tastant ligands or a composition comprising one or more putative tastant ligands, wherein the at least two fragments act as a hetero-oligomeric complex; and

(ii) detecting binding of a tastant ligand to the at least two fragments thereby indicating the one or more putative tastant ligands have binding specificity for said G protein-coupled receptors active in taste signaling.

40. The assay of claim 39, wherein binding is detected by displacement of a radiolabeled known binding ligand.

41. The assay of claim 40, wherein said known binding ligand is an antibody or antibody fragment having binding specificity to said G protein-coupled receptor.

42. A biochemical assay for identifying tastant ligands having binding specificity for G protein-coupled receptors active in sweet taste signaling, comprising:

1 (i) contacting at least two fragments according to claim 20 with a preparation of G proteins and GTP γ S, and one or more putative tastant ligands or a composition comprising one or more putative tastant ligands, wherein said at least two fragments act as a hetero-oligomeric complex; and

5 (ii) detecting binding of a tastant ligand having binding specificity for said G protein-coupled receptors active in taste signaling by measuring the binding of GTP γ S to the G protein.

10 43. A method for enhance surface expression of integral plasma membrane proteins comprising fusing a PDZ-interacting peptide to an integral plasma membrane protein to form a heterologous protein, and expressing the heterologous protein in a cell.

15 44. The method of claim 43 wherein the PDZ-interacting peptide consists essentially of the amino acid sequence of SEQ ID NO 10.

20 45. The method of claim 43, wherein the integral plasma membrane protein is a G-protein coupled receptor.

25 46. The method of claim 43, wherein the PDZ-interacting peptide is fused to the C-terminus of the G-protein coupled receptor.

47. The method of claim 45, wherein the G-protein coupled receptor is selected from the group consisting of olfactory receptors and taste receptors.

25 48. The method of claim 45, wherein the G-protein coupled receptor is a receptor having an amino acid sequence selected from the group consisting of SEQ ID NOS 2, 4, and 6.

30 49. An expression vector comprising an isolated nucleic acid molecule encoding for a G-protein coupled receptor polypeptide fused to a surface expression facilitating sequence having an SEQ ID NO 10, wherein said vector is selected from

the group consisting of mammalian vectors, bacterial plasmids, bacterial phagemids, mammalian viruses and retroviruses, bacteriophage vectors and linear or circular DNA molecules capable of integrating into a host cell genome.

5 50. A host cell transfected with at least one of the expression vectors of claim 49, wherein said host cell expresses the encoded G protein-coupled receptor polypeptides on the surface of said host cell.

10 51. An isolated nucleic acid molecule selected from the group consisting of:

15 (i) a genomic DNA sequence consisting essentially of a nucleic acid sequence coding for a T1R mammalian G protein-coupled receptor polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs 2, 4, and 6;

20 (ii) a genomic DNA sequence consisting essentially of a nucleic acid sequence coding for a T1R mammalian G protein-coupled receptor polypeptide having an amino acid sequence that is at least about 40% identical to the amino acid sequence selected from the group consisting of SEQ ID NOs 2, 4, and 6;

25 (iii) a genomic DNA sequence consisting essentially of a sequence coding for a T1R mammalian G protein-coupled receptor polypeptide comprising a consensus sequence selected from the group consisting of SEQ ID NOs 8 and 9, and sequences having at least about 75% identity to SEQ ID NOs 8 or 9;

30 (iv) a cDNA sequence coding for a T1R mammalian G protein-coupled receptor polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs 2, 4, and 6;

35 (v) a cDNA sequence coding for a T1R mammalian G protein-coupled receptor polypeptide comprising a consensus sequence selected from the group consisting of SEQ ID NOs 8 and 9, and sequences having at least about 75% identity to SEQ ID NOs 8 or 9;

40 (vi) a cDNA sequence selected from the group consisting of SEQ ID NOs 1, 3, 5, and 7;

RECEIVED
SEARCHED
INDEXED
MAILED

(vii) a cDNA sequence having at least about 50% sequence identity to a sequence encoding a T1R mammalian G protein-coupled receptor polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs 2, 4, and 6;

5 (viii) a cDNA sequence having at least about 50% identity to a sequence selected from the group consisting of SEQ ID 1, 3, 5, and 7;

(ix) a variant of a nucleotide sequence encoding a T1R G protein-coupled receptor polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS 2, 4, and 6, containing at least one conservative 10 substitution in a T1R mammalian G protein-coupled receptor polypeptide coding region; and

(x) a variant of a cDNA sequence selected from the group consisting of SEQ ID NOs 1, 3, 5, and 7, containing at least one conservative substitution.

15 52. An isolated RNA molecule transcribed from the isolated nucleic acid molecule of claim 51.

53. An isolated nucleic acid molecule that hybridizes to the nucleic acid molecule of claim 51 under stringent hybridization conditions.

20 54. An isolated nucleic acid molecule that hybridizes to the nucleic acid molecule of claim 51 under moderate hybridization conditions.

55. An isolated fragment of the nucleic acid molecule of claim 51 that is at 25 least about 20 to 30 nucleotide bases in length.

56. A chimeric or fused nucleic acid molecule, wherein said chimeric or fused nucleic acid molecule comprises at least part of the coding sequence contained in the nucleic acid molecule of claim 51, and at least part of a heterologous coding 30 sequence, wherein transcription of said chimeric or fused nucleic acid molecule results in a single chimeric nucleic acid transcript.

57. The chimeric or fused nucleic acid molecule of claim 56, wherein said heterologous coding sequence is from a sequence encoding a different G protein-coupled receptor.

5 58. The chimeric or fused nucleic acid molecule of claim 56, wherein said heterologous coding sequence is a sequence that facilitates expression of said mammalian G protein-coupled receptor polypeptide on the surface of a cell.

10 59. The chimeric or fused nucleic acid molecule of claim 58, wherein said heterologous coding sequence is SEQ ID NO 10.

15 60. The chimeric or fused nucleic acid molecule of claim 56, wherein said heterologous coding sequence is from a gene encoding green fluorescent protein or other detectable marker gene.

15 61. An isolated nucleic acid molecule consisting essentially of a nucleic acid sequence coding for a mammalian G protein-coupled receptor having an amino acid sequence selected from the group consisting of SEQ ID NOs 2, 4, and 6.

20 62. An isolated RNA molecule transcribed from the isolated nucleic acid molecule of claim 61.

25 63. An isolated nucleic acid molecule that hybridizes to the nucleic acid molecule of claim 61 under stringent hybridization conditions.

64. An isolated nucleic acid molecule that hybridizes to the nucleic acid molecule of claim 61 under moderate hybridization conditions.

30 65. An isolated fragment of the nucleic acid molecule of claim 61 that is at least about 20 to 30 nucleotide bases in length.

66. A chimeric or fused nucleic acid molecule, wherein said chimeric or fused nucleic acid molecule comprises at least part of the coding sequence contained in the nucleic acid molecule of claim 61, and at least part of a heterologous coding sequence, wherein transcription of said chimeric or fused nucleic acid molecule results in a single chimeric nucleic acid transcript.

10 67. The chimeric or fused nucleic acid molecule of claim 66, wherein said heterologous coding sequence is from a sequence encoding a different G protein-coupled receptor.

15 68. The chimeric or fused nucleic acid molecule of claim 66, wherein said heterologous coding sequence is a sequence that facilitates expression of said mammalian G protein-coupled receptor polypeptide on the surface of a cell.

20 69. The chimeric or fused nucleic acid molecule of claim 68, wherein said heterologous coding sequence is SEQ ID NO 10.

70. The chimeric or fused nucleic acid molecule of claim 66, wherein said heterologous coding sequence is from a gene encoding green fluorescent protein or other detectable marker gene.

25 71. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs 1, 3, 5, and 7.

72. An isolated RNA molecule transcribed from the isolated nucleic acid molecule of claim 71.

30 73. An isolated nucleic acid molecule that hybridizes to the nucleic acid molecule of claim 21 under stringent hybridization conditions.

74. An isolated nucleic acid molecule that hybridizes to the nucleic acid molecule of claim 71 under moderate hybridization conditions.

75. An isolated fragment of the nucleic acid molecule of claim 71 that is at least about 20 to 30 nucleotide bases in length.

5 76. A chimeric or fused nucleic acid molecule, wherein said chimeric or fused nucleic acid molecule comprises at least part of the coding sequence contained in the nucleic acid molecule of claim 71, and at least part of a heterologous coding sequence, wherein transcription of said chimeric or fused nucleic acid molecule results in a single chimeric nucleic acid transcript.

10 77. The chimeric or fused nucleic acid molecule of claim 76, wherein said heterologous coding sequence is from a sequence encoding a different G protein-coupled receptor.

15 78. The chimeric or fused nucleic acid molecule of claim 76, wherein said heterologous coding sequence is a sequence that facilitates expression of said mammalian G protein-coupled receptor polypeptide on the surface of a cell.

20 79. The chimeric or fused nucleic acid molecule of claim 78, wherein said heterologous coding sequence is SEQ ID NO 10.

80. The chimeric or fused nucleic acid molecule of claim 76, wherein said heterologous coding sequence is from a gene encoding green fluorescent protein or other detectable marker gene.

25 81. A nucleic acid molecule comprising the isolated nucleic acid molecule of claim 71 operably linked to a heterologous promoter that is either regulatable or constitutive.

30 82. The nucleic acid molecule of claim 81, wherein said regulatable promoter is inducible under specific environmental or developmental conditions.

83. An isolated variant molecule comprising a nucleotide sequence encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs 2, 4, and 6, containing at least one conservative substitution in a coding region.

5

84. An isolated RNA molecule transcribed from the isolated variant molecule of claim 83.

85. An isolated nucleic acid molecule that hybridizes to the variant 10 molecule of claim 83 under stringent hybridization conditions.

86. An isolated nucleic acid molecule that hybridizes to the variant molecule of claim 83 under moderate hybridization conditions.

15 87. An isolated fragment of the variant molecule of claim 83 that is at least about 20 to 30 nucleotide bases in length.

20 88. A chimeric or fused nucleic acid molecule, wherein said chimeric or fused nucleic acid molecule comprises at least part of the coding sequence contained in the variant molecule of claim 83, and at least part of a heterologous coding sequence, wherein transcription of said chimeric or fused nucleic acid molecule results in a single chimeric nucleic acid transcript.

25 89. The chimeric or fused nucleic acid molecule of claim 88, wherein said heterologous coding sequence is from a sequence encoding a different G protein-coupled receptor.

30 90. The chimeric or fused nucleic acid molecule of claim 88, wherein said heterologous coding sequence is a sequence that facilitates expression of said mammalian G protein-coupled receptor polypeptide on the surface of a cell.

91. The chimeric or fused nucleic acid molecule of claim 90, wherein said heterologous coding sequence is SEQ ID NO 10.

92. The chimeric or fused nucleic acid molecule of claim 88, wherein said 5 heterologous coding sequence is from a gene encoding green fluorescent protein or other detectable marker gene.

93. A cDNA molecule having the same nucleic acid sequence as the coding region of the variant DNA molecule of claim 83.

10

94. A nucleic acid molecule comprising the cDNA molecule of claim 93 operably linked to a heterologous promoter that is either regulatable or constitutive.

15

95. The nucleic acid molecule of claim 94, wherein said regulatable promoter is inducible under specific environmental or developmental conditions.

96. The isolated nucleic acid molecule of claim 51, wherein said nucleic acid encodes a G protein-coupled receptor polypeptide that is active in sweet taste signaling in rat, mouse, or human.

20

97. An expression vector comprising an isolated nucleic acid molecule of claim 51, wherein said vector is selected from the group consisting of mammalian vectors, bacterial plasmids, bacterial phagemids, mammalian viruses and retroviruses, bacteriophage vectors and linear or circular DNA molecules capable of integrating 25 into a host cell genome.

98. A host cell transfected with at least one of the expression vectors of claim 97, wherein said host cell expresses the encoded G protein-coupled receptor polypeptides on the surface of said host cell.

30

99. A nucleic acid array comprising at least about 20 to 30 nucleotides of at least one of the isolated nucleic acid molecules of claim 51, wherein the at least one nucleic acid molecules are linked covalently or noncovalently to a solid phase support.

Add a^3

卷之三